Paul Holliday

WEST AFRICAN
COCOA RESEARCH INSTITUTE
Technical Bulletin No. 7

THE VIRUSES OF CACAO

J. M. THRESH & T. W. TINSLEY

COMMONWEALTH MYCOLOGICAL INSTITUTE
- 6 OCT 1970

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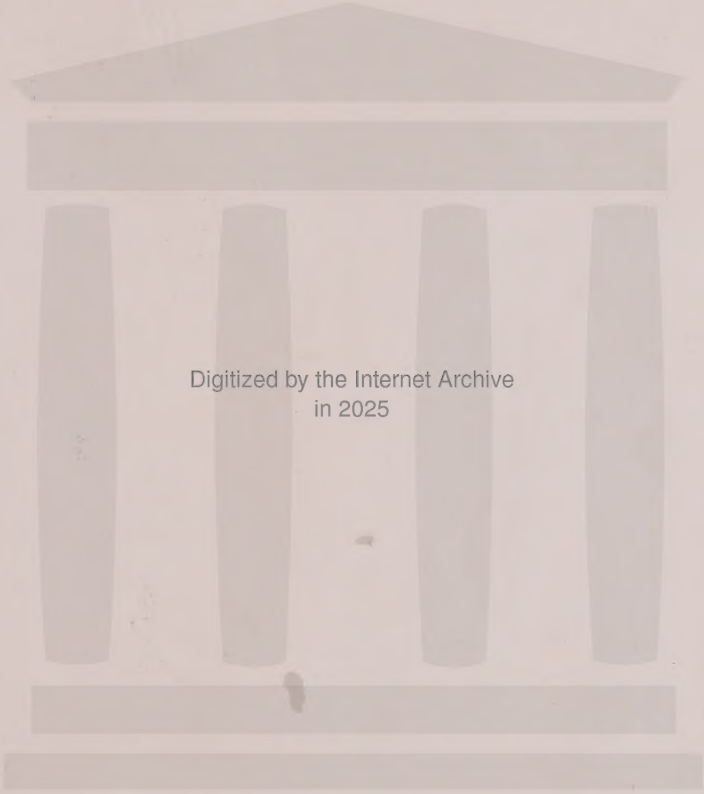

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Conspicuous chlorotic vein-banding of cacao leaves caused by a virulent isolate of cacao swollen shoot virus from Western Nigeria

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W.A.C.R.I. Sub-Station, Ibadan, Nigeria

Published on behalf of
The West African Cocoa Research Institute
by the
Crown Agents for Oversea Governments and Administrations,
4, Millbank, London S.W.1

1959

Printed by Eyre and Spottiswoode Limited
Her Majesty's Printers
at The Chiswick Press, London

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* This review summarises published papers which appeared before 1st June, 1958, and also refers to unpublished papers and reports which were seen in manuscript.

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INTRODUCTION

In 1936 cacao trees in the New Juaben district of the Eastern Province of Ghana were affected by a disease which caused swellings on the branches (Steven, 1936). Posnette (1940) established that this swollen shoot condition was caused by a virus and realised that several viruses or virus strains were involved in the numerous outbreaks (Posnette, 1941, 1943a). Later it was found, not only that different trees in the same outbreak may be infected by viruses differing considerably in the severity of their effects and in symptom expression (Posnette & Todd, 1951, 1955), but also that symptomologically distinct viruses may occur together in the same tree (Posnette & Todd, 1955). A similarly complex situation occurs in Ivory Coast (Mangenot, Alibert & Basset, 1946) and Nigeria (Thresh, 1958c). Cacao viruses also occur in Trinidad (Posnette, 1944), Ceylon (Peiris, 1953) and Sierra Leone and possibly in Colombia (Ciferri, 1948), Venezuela (Posnette & Palma, 1944) and Java (Thung, unpublished information), but there have been only limited investigations in these countries.

The present paper summarises published and unpublished information on the numerous isolates which have been made from cacao. The available criteria for determining the relationships between them are also discussed and used to propose a provisional classification.*

* The cacao literature contains various references to viruses, strains, forms and types. At the risk of repetition we have in general preferred the non-committal term isolate, although evidence is presented finally that there are at least three distinct viruses in West Africa, each with various strains.

GENERAL FEATURES OF CACAO VIRUSES AND CRITERIA FOR THEIR CLASSIFICATION

The Symptoms Produced on Cacao

Virus isolates from cacao have been distinguished by the symptoms produced on plants infected either by grafts or by the mealybug vectors feeding on cuttings, seedlings or seeds. The production of symptoms is influenced by inoculation method, age and variety of host and by the inoculum. In West Africa it is usual to use seedling Amelonado cacao and Posnette (1947b) found that beans were the most convenient material to inoculate. Their reaction to infection was as uniform as that of clonal material (Posnette, 1947a), but seedlings of other cacao types are more variable (Posnette & Todd, 1951; Dale, 1957b) and different Trinidad clones do not all produce similar symptoms when inoculated with the same isolate (Baker & Dale, 1947).

Infected plants must be observed for several months to follow the full sequence of symptom development, because different isolates may cause similar symptoms at certain stages. Symptom records must also be taken frequently when the plants are flushing, or the transient symptoms are missed.

Viruses cause a range of symptoms in cacao and these are discussed in the following sections. Some of the symptoms are only caused by virus infection, while others are of less diagnostic value and may also result from other causes.

Swellings

Cacao branches are of two types, the fan branches with alternate leaves and chupons with spiral phyllotaxy. Swellings caused by virus infection develop on branches of either type, at the nodes, internodes or apex (Plate 3, Figure 2). They tend to be most numerous and conspicuous on rapidly growing shoots (Posnette, 1941). Some isolates cause stem and root swellings more readily than others and some never cause swellings or only affect roots (Attafuah, 1957). The significance of this is discussed later.

Mangenot *et al.* (1948) and Knight & Tinsley (1958) found that swellings were caused by localised increases in the amount of secondary phloem and xylem, and the tracheids in affected areas were unusually large and the cambium tissue unusually wide.

Leaf symptoms

Plants infected with cacao viruses show characteristic symptoms on leaves produced after inoculation (Posnette, 1941). Transient symptoms appear on the flush leaves and are replaced by symptoms that persist until abscission. The different symptoms are apparently distinct manifestations of virus activity, as transient symptoms are not always followed by permanent ones and symptoms may develop on hardening leaves which were previously normal.

The transient leaf symptoms are caused by an abnormal accumulation of anthocynanins in the mesophyll cells along the tissues of the veinlets, to give a reticulate pattern of red vein banding (Knight & Tinsley, 1958). The intensity and extent of this depends upon the interaction between virus and host and also on the stage of infection, but may be limited to individual veinlets or the leaf may be extensively mottled (Kirkpatrick, 1950). This distribution differs from that in healthy tissues, where the pigment varies according to the type of cacao but is uniformly distributed.

The red vein banding and mottle symptoms usually appear when affected leaves begin to expand and they may intensify for a time, but fade within a few days as the leaves mature and develop more chlorophyll. However, the anthocyanin persists in some leaves, causing a dark red-green mottle of the tissues alongside affected veins. Transient symptoms are most conveniently seen in unpigmented Amelonado cacao; an additional advantage of this material compared with heavily pigmented types.

The permanent leaf symptoms usually take the form of clearings or chlorosis on or alongside the veins (Plates 4 and 5) and the mesophyll cells in affected areas do not differentiate to form intercellular spaces, and the chloroplasts are immature (Knight & Tinsley, 1958). The vein banding depends upon the virus and stage of infection; it may be restricted to the veinlets, giving the speckling symptom caused by many mild isolates (Plate 5, Figure 2), or it may extend along the third and fourth order veins, giving angular flecks (Plate 5, Figure 3). These are often the first symptoms produced on plants which have not already shown transient symptoms. Other leaves show a more extensive chlorosis or one forming a characteristic pattern along the principal veins (Plate 5, Figure 4).

Leaf symptoms usually occur over the entire lamina, but occasionally the severe chlorosis is restricted to only one side of the midrib (Plate 5, Figure 1). Leaves thus affected are usually distorted and crinkled. These symptoms are caused by the most virulent isolates in West Africa, and are apparently caused by differences in the rate at which normal and affected tissues expand and differentiate.

Some virus isolates are unusual in causing a necrosis of the minor veins (Plate 2, Figures 1 & 2). This may be extensive, causing affected leaves to die, or may be limited to certain veinlets which develop a chlorotic halo (Thresh, 1958b). Subsequent leaves are produced with translucent distorted patches somewhat similar to the ones caused by isolates from Ivory Coast and Nigeria. These symptoms are due to a failure of the tissues to differentiate and form chloroplasts (Mangenot, *et al.*, 1946).

Definite symptom phases can usually be distinguished after inoculation. Thus one of the Ghana isolates produces conspicuous symptoms on only one flush and most subsequent growth seems normal. Other isolates show a distinct acute phase of infection (sometimes with severe necrosis of the leaves and shoots), followed by a chronic or recovery phase with limited symptoms. Early leaf symptoms tend to be conspicuous and severe, whereas later symptoms are mild, with relatively simple patterns along the main veins of normal sized leaves. Mild isolates often produce symptomless flushes at this chronic stage and the symptoms which do appear are usually slight.

The symptom phases may be due to changes in virus concentration, to differences in reaction of the leaves according to the stage at which they are invaded by virus, or to changes in the tolerance of plants to infection with age. Qualitative changes in virus seem not to be involved and plants infected in transmission tests made at various intervals after infection show similar symptoms.

Pod symptoms

Trees infected with some virulent isolates produce abnormal pods which are characteristically small and rounded (Plate 3, Figure 1) (Posnette, 1943b; 1947a; Carter, 1956). Virus can be recovered from these pods and from normal ones borne on infected trees, but not from the beans, although these are often small and grow into weak plants.

Immature and unpigmented pods on infected trees may also show a characteristic dark green mottle, which may be pink on exposed pods (Posnette, 1943b; 1947a). Indeed, a transient pod mottle may be the first symptom on trees which have made little or no vegetative growth after inoculation. Red blotches have been noticed on the yellow pods of certain I.C.S. clones infected with one of the Trinidad isolates (Baker & Dale, 1947).

Effects on growth and yield

Goodall (1949) observed that cacao seedlings infected with isolates from Ghana and Nigeria had smaller leaf areas, and lower dry

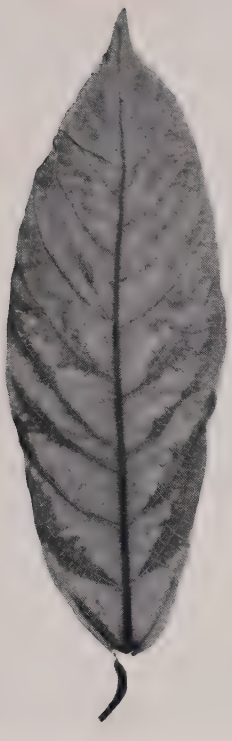


Fig. 1. Red mottle on a flush leaf caused by an isolate from Kpeve, Ghana.

Fig. 2. Vein clearing and banding caused by an isolate from Alaparun, Nigeria.

PLATE I. CACAO MOTTLE LEAF VIRUS

Fig. 3. *Adansonia digitata* L. an alternative host of cacao mottle leaf virus in Ghana.

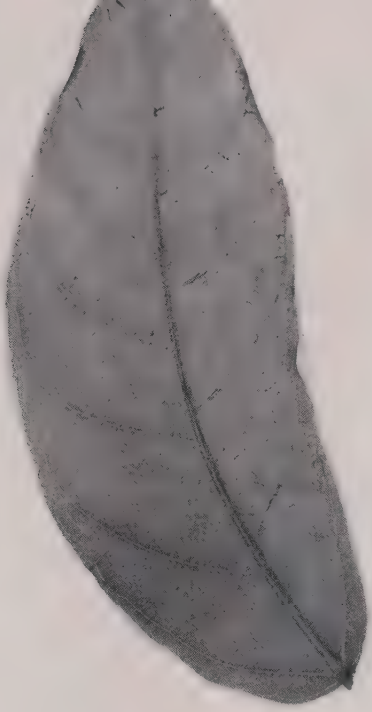


Fig. 1. Veinal necrosis on a leaf produced in the acute stage of infection.

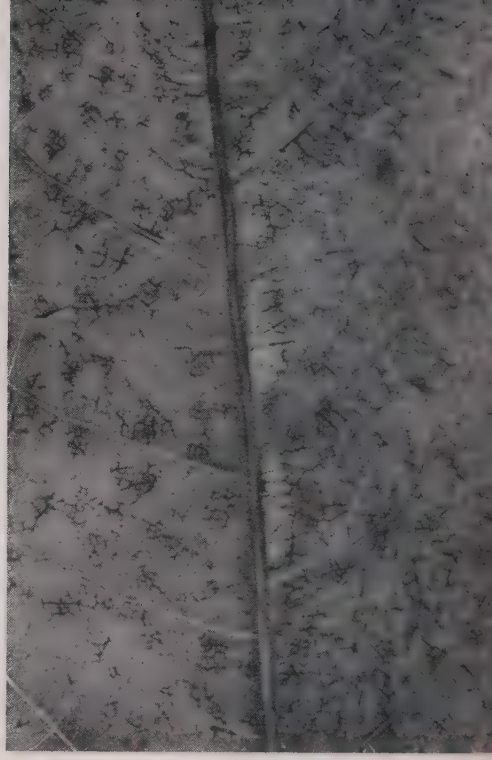


Fig. 2. Veinal necrosis magnified.

PLATE 2. CACAO NECROSIS VIRUS

Fig. 3. Translucent lesions on a leaf produced in the chronic stage of infection.

Fig. 4. Translucent lesions magnified.

weights, relative growth rates and net assimilation rates than comparable healthy controls. Infection also reduced the water content of the plants and caused necrosis of the fine roots. Similar effects are caused by other isolates from Ghana (Posnette, 1948) and Nigeria (Posnette, 1950a; Lister & Thresh, 1955; Thresh, unpublished information). Moreover, young plants which are protected from insect attack sometimes die back after infection with virulent virus and two Nigerian ones cause extensive defoliation and commonly kill plants infected as beans.

Infected trees are usually less vigorous than healthy ones in Ghana (Crowdy & Posnette, 1947; Posnette & Todd, 1951, 1955), Nigeria (Lister & Thresh, 1957a) and Trinidad (Baker & Dale, 1947; Cope, 1953) and yield usually declines with the length of time trees have been infected, at a rate depending upon growing conditions. Where these are unsatisfactory and capsid attack is severe the trees deteriorate rapidly and may die. Elsewhere the trees may survive for years, particularly when infected with mild strains.

Virus infection may cause premature defoliation and expanding leaves may turn almost white, degenerate and fall at the time chlorophyll normally accumulates. Persistent leaves may be symptomless, but are often chlorotic and abnormally small (Posnette, 1943b). Virus may also disturb the normal growth rhythm and infected trees may be dormant when healthy ones are flushing vigorously and *vice versa* (Posnette, 1943b). Moreover, growth flushes on infected trees are less vigorous than on healthy ones and the internodes tend to be short and the leaves few.

Symptoms as criteria of relationship

More than 100 isolates have been distinguished by the symptoms they cause in cacao and this number could probably be increased if finer differences in symptom expression were considered and relatively stable isolates obtained by repeated single insect transfers. Uniform indicator or resistant clones might also be valuable, particularly if they give differential reactions (cf. Holmes, 1958). However, symptoms are of limited value in indicating virus relationships (Bawden, 1950; Smith, 1957), particularly with isolates from cacao. Thus two from Offa Igbo and New Juaben cause similar symptoms on cacao, yet do not protect against each other, suggesting no close relationship. By comparison, the mild and severe isolates from New Juaben cause dissimilar symptoms but show complete protection.

Transmission

Grafting methods

Cacao viruses were first transmitted experimentally by grafts,

using patches of bark from hardened stems (Posnette, 1940). Successful transmissions were made subsequently by budding, inarching and cleft grafts and by grafting on to embryos (Archibald 1954).

Sap transmissions

None of the virus isolates has been transmitted by sap inoculation despite numerous attempts by several workers using different techniques. These repeated failures may be due to the inhibitory effects of polyphenolic materials and/or mucilage liberated on macerating leaves. However, these are probably not the only factors involved, as Kassanis (in Bawden, 1951) readily transmitted a tobacco necrosis virus to and from cacao. The inability to transmit any of the cacao viruses by sap inoculation means that there is no information on their properties *in vitro* and it is hardly surprising that attempts to produce antisera against various Ghana isolates failed (Tinsley, unpublished information).

Insect transmissions

Cacao viruses are not apparently seed or soil borne (Posnette, 1947a) or spread by contact between plants. Consequently, they are usually spread by the movement of the mealybug vectors, which have transmitted viruses from cacao in West Africa (Posnette & Strickland, 1948; Posnette, 1950; Posnette & Robertson, 1950), Trinidad (Kirkpatrick, 1950) and Ceylon (Carter, 1956). Indeed, mealybugs have transmitted all the cacao viruses with which they have been adequately tested, except for one group from south west Nigeria, where outbreaks appear to spread although no vector has been found.

The probability of mealybugs transmitting a virus obtained from Mampong in Ghana increased with the number of vectors used and with the time spent on the source plants and test plants up to optimum periods of several hours and 50 minutes respectively (Posnette & Robertson, 1950; Dale, 1957a). Previously starved insects transmitted virus more often than those unstarved, after feeding on infected plants for less than 10 hours. Moreover, virus persisted in feeding insects for less than one hour, but up to 36 hours in starved adults. Similar results were obtained in Trinidad (Kirkpatrick, 1950) and there is at present no basis for classifying the isolates according to the conditions under which they are transmitted.

Vector specificity and its importance in classification

Some mealybug species transmit certain isolates, but not others and this suggests a possible apprach to classification, as with other viruses. One difficulty is to obtain enough mealybugs, as some of the key

species are difficult to breed. Moreover, transmission depends partly upon virus availability, which in turn depends on the feeding conditions, the source plants and the particular virus isolate employed. Thus experiments are difficult to standardise, although some of the variables are eliminated by using sources simultaneously infected with two isolates (Posnette, 1950b).

Most of the vector experiments have been made with viruses and mealybugs from Ghana, Nigeria and Ivory Coast (Posnette, 1950b), but some were done elsewhere (Kirkpatrick, 1950; Carter, 1956). The available results have recently been discussed (Martini, 1958).

Host Range

The host range of certain West African isolates was investigated at Tafo (Posnette, Robertson & Todd, 1950; Dale & Attufuah, 1957; Tinsley & Wharton, 1958), and the one from New Juaben infected more species than any of the others. With one exception its host range included all the species susceptible to other isolates. The susceptibility of the different species and their reaction to infection varied considerably and the isolates fell into three groups according to their ability to infect key species (Table I).

Susceptible species belong to the Sterculiaceae, Bombacaceae and Tiliaceae of the Tiliales and to the Malvaceae of the Malvales. None of the many other tested species in a wide range of families has yet been infected, and the host range of cacao viruses is apparently limited. However, generalisations are not justified because there is no information on the host range of the Nigerian isolates which cause vein necrosis, or of those from Ivory Coast and areas outside West Africa.

Strains of many viruses have been differentiated by their host range and this approach may be valuable in classification. Unfortunately, seeds are often not available and mealybugs must be used to inoculate plants and demonstrate virus. This is an important complication as mealybugs feed with difficulty or not at all on certain hosts and conditions are difficult to standardise. Thus virus concentration and availability may mask qualitative differences between isolates and a resistant host may be infected by strains which are readily transmitted by mealybugs, but is apparently immune to others. Moreover, hosts may sometimes be highly susceptible, but apparently resistant or immune at others, depending upon the feeding behaviour of the mealybugs and on the virus source. This may explain the apparent immunity of *Cola chlamydantha* K. Schum. to the New Juaben isolate in some experiments (Posnette *et al.*, 1950), but not others (Tinsley & Wharton, 1958).

TABLE I

*The Susceptibility of Sixteen Species of the Tiliales to Eight Isolates of Virus from Cacao in West Africa**

Species	*Viruses*							
	1	2	3	4	5	6	7	8
STERCULIACEAE								
†*Cola chlamydantha* K. Schum.	+	+	+	–	–	–	–	–
†*Cola gigantea* A. Chev. var. *glabrescens* Brenan & Keay	+	+	+	+	–	–	–	–
†*Cola lateritia* K. Schum. *maclaudi* (A. Chev.) Brenan & Keay	+	+	+	+		–	+	+
Herrania balaensis Preuss	+	+	+	+	–	–	+	+
Herrania sp. 'A' (indet.)	+	–	+	+	–	+	–	+
Herrania sp. 'B' (indet.)	+	+	+	+	+	+	–	+
†*Pterygota macrocarpa* K. Schum.	+	–	+	–	+	+	–	–
†*Sterculia rhinopetala* K. Schum.	+	–	+	+	+	+	+	
†*Sterculia tragacantha* Lindl.	+	+	+	+	–	+	–	–
Theobroma bicolor Hum. & Bonpl.	+	+	+	+	+	+	+	–
Theobroma speciosa Spreng.	+	+	+	+	+	–	–	.
Theobroma grandiflora K. Schum.	+	+	–	–			–	–
Theobroma angustifolia DC.	+	+	+	+	–	–	–	–
Theobroma obovata Bern.	+	+	.	+		.	.	.
BOMBACACEAE								
†*Bombax buonopozense* P. Beauv.	+	+	+	+	+	–	–	–
†*Ceiba pentandra* (L.) Gaertn.	+	+	+	+	+	+	+	–

Cacao virus isolates from:

1. New Juaben (Ghana)
2. Offa Igbo (Nigeria)
3. Bosumuoso (Ghana)
4. Bosumtwe (Ghana)
5. Kpeve (Togoland)
6. Mampong (Ghana)
7. Alaparun (Nigeria)
8. Bisa (Ghana)

* Table from Tinsley & Wharton (1958) omitting records for six species which were susceptible to all eight viruses.

† Indigenous to West Africa.

\+ Species susceptible.

– Species not susceptible.

. Material not available for testing.

Protection Phenomena

Plants already infected with one virus may be totally or partially immune from the effects of the same or related strains. Indeed, interference has been used as a basic criterion for determining relationships (Price, 1940; Bawden, 1950; Bennett, 1953). Definite protection indicates close relationships, but protection may be incomplete or even absent between related strains (Bawden & Kassanis, 1951; Harrison, 1958).

In routine protection tests with cacao viruses, one-year-old seedlings were infected with an isolate and subsequently challenged with a second one three months later or when symptoms had appeared. The number and size of the grafts were not usually specified in the work already published and it is not clear whether

the plants were cut back at any stage, although these factors affect the establishment of the challenging virus (Posnette & Todd, 1955).

Graft patches probably act as a source of inoculum for weeks or even months, whereas there is probably less time for mealybugs to introduce the challenging virus. This suggests that grafting methods are less likely to detect distant relationships than mealybug transmissions, although this possibility has not been investigated. However, the Bisa isolate did not protect plants from subsequent infection by New Juaben applied by grafts, but partial protection occurred in the field (Crowdy & Posnette, 1947).

Results of protection tests are not always reciprocal and each isolate should be used in turn for the first inoculation. This is only practicable when symptoms caused by the challenging strain are recognisable if they occur on plants already infected with the first.

TABLE IIa

*The Results of Protection Tests with Isolates from the Western Province of Ghana**

Challenging Isolate / Basal Isolate	Bosumuoso I	Bosumuoso II	Datano	Jamesi	Punekrom	Suhuma	Suranwo	Bakukrom	Aiyiboso	Amafie	Eddu Suazo	Wiasi
Bosumuoso I	/	.										
Bosumuoso II	.	/	.			.	.		.			
Datano		.	/	.		.	.		.	.	.	
Jamesi			.	/		.		.				
Punekrom					/							
Suhuma		.	.	.		/	.		.	.		
Suranwo		.	.			.	/			.	.	
Bakukrom				.		.	.	/	.	.		
Aiyiboso		.	.			.	.	.	/	.		
Amafie			.			.		.	.	/		
Eddu Suazo			.				.				/	.
Wiasi											.	/

* Date from Tinsley and Todd (unpublished information).
. Symptoms of the challenging virus produced without apparent interference.

TABLE IIb

*The Results of Protection Tests with some Nigerian Viruses**

Challenging Isolate / Basal Isolate	Alaparun	Offa Igbo I	Offa Igbo II	Olanla I	Olanla II	Ilesha	Asalu
Alaparun	/	.	.	.	.	.	.
Offa Igbo I	.	/	+	.	.	.	.
Offa Igbo II	.	+	/	.	.	.	.
Olanla I	.	.	.	/	+	+	.
Olanla II	.	.	.	+	/	+	.
Ilesha	.	.	.	+	+	/	.
Asalu	.	.	.	.	.	.	/

* Data from Tinsley (unpublished information).
. Symptoms of the challenging virus produced without apparent interference.
\+ Symptoms of the challenging virus not noted.

Thus mild isolates or those causing leaf symptoms only are most conveniently used as the first inoculum and challenged with others causing severe or characteristic symptoms. Even so, relatively few of the many possible tests have been done. Most of them involved pairs of isolates from different localities and the symptoms of the challenging virus were produced normally, apparently uninfluenced by the first inoculation. Other isolates caused such similar symptoms that those of the challenging virus were not recognised on plants already infected with the basal one and the interpretation of these tests was equivocal. The relatively few tests in which mild isolates protected plants from subsequent infection with virulent ones were of greater importance as they indicated close relationships. Additional interference phenomena were noted between pairs of isolates from Ghana and when the ones from Mampong and New Juaben occurred together they caused unique symptoms (Posnette, 1950b). Furthermore, the isolate from Bisa partially protected plants from subsequent infection with the New Juaben one, which then caused unusually mild symptoms and these only after a long latent period.

Most protection tests have involved mild isolates from New Juaben combined with others from Ghana, but some work has been done with isolates from Ivory Coast and Nigeria. In the most recent work the isolates were grouped geographically and attempts made to

make all the combinations between those in each group and between representative isolates from each (Tinsley, 1953). Tables IIa & IIb summarise the contrasting results of tests with isolates from the Western Province of Ghana and others from Western Nigeria. Close relationships are indicated only between some of the Nigerian isolates.

THE DIFFERENCES BETWEEN VIRUSES FROM DIFFERENT LOCALITIES

Within West Africa, virus has been reported from numerous localities in Ghana, Ivory Coast, Nigeria and Sierra Leone. Consequently many outbreaks are available for study and detailed observations have been made in many localities and numerous isolates taken. Naturally infected trees usually show leaf symptoms and swellings. However, only swellings have been found at some outbreaks and at others only leaf symptoms. These vary and may be so mild and inconspicuous that the trees seem healthy, or so extensive and severe that infected trees show obvious defoliation and die-back.

These field observations indicate that many different viruses or strains occur; a conclusion substantiated by the observation that uniform seedlings usually show symptoms which resemble those on the field source, and are consistent on successive transfers. Some isolates have been described in detail and with photographs (Posnette, 1947a), while others have been briefly mentioned in WACRI Quarterly, Annual and unpublished reports. The symptoms caused by a representative sample of West African isolates are summarised in Table III. The present section merely indicates the range of isolates described from West Africa, Trinidad and Ceylon. In these countries there have been surveys of the extent of infection, but no detailed assessment of the relative importance of the different isolates and the search for unusual symptom variants may have been misleading.

Ghana

Early work in the Eastern Province suggested that most of the infected trees contained the virulent New Juaben virus, which causes swellings and conspicuous leaf symptoms. However, dissimilar isolates occur and one from Bisa caused only swellings and partially protected plants from subsequent infection with the New Juaben virus. Other isolates from Nkawkaw, Mampong and Nsaba failed to protect against each other or against the New Juaben virus. The

Table III

*A Summary of the Symptoms caused by a Representative Selection of Cacao Virus Isolates from Nigeria and Ghana**

Isolate	Country	Swellings	Pod Mottle	Defoliation on Seedlings	Leaf Symptoms								Distinct Acute and Chronic Phases
					Red Vein Banding	Red Mottle	Chlorotic Mottle	Chlorotic Vein Banding		Translucent Patches	Distortion or Crinkle	Veinal Necrosis	
								Mild	Severe				
Cacao Necrosis Virus													
Omi Aboderin	Nigeria	–	–	+	–	–	–	–	–	+	+	+	+
Asalu	Nigeria	–	–	+	–	–	–	–	–	+	+	+	+
Cacao Mottle Leaf Virus													
Kpeve	Ghana	–	–	+	+	+	+	–	–	+	–	–	–
Alaparun	Nigeria	–	–	+	+	+	+	–	–	+	–	–	–
Baobab virus	Ghana	–	–	+	+	+	+	–	–	+	–	–	–
Cacao Swollen Shoot Viruses													
Bisa	Ghana	+	–	–	+	–	–	+	–	–	–	–	–
Iweke	Nigeria	+	–	–	+	–	–	+	–	–	–	–	–
I.N.A.	Nigeria	+	–	–	+	–	–	+	–	–	–	–	–
Balogun	Nigeria	–	–	–	+	–	–	+	–	–	–	–	–
Abuku	Nigeria	–	–	+	+	–	–	+	+	–	+	–	–
Ilesha	Nigeria	+	+	–	+	+	–	+	–	+	–	–	–
Olanla	Nigeria	+	+	–	+	+	–	+	–	+	–	–	–
Nkawkaw	Ghana	+	–	–	+	+	–	+	–	+	–	–	–
Elepo	Nigeria	+	–	–	+	–	–	+	–	–	–	–	–
Offa Igbo	Nigeria	+	–	–	+	–	–	+	+	–	–	–	+
New Juaben	Ghana	+	+	+	+	–	–	+	+	–	+	–	+
Egbeda	Nigeria	+	+	+	+	–	–	+	+	–	+	–	+
Bosumtwe	Ghana	+	–	–	+	–	–	+	+	+	–	–	+
Bosumuosu	Ghana	+	+	+	+	–	–	+	+	–	–	–	+
Mampong	Ghana	+	–	–	+	–	–	+	–	–	–	–	+

* The basis for ascribing the isolates to three distinct viruses is discussed in a later section.
\+ Symptoms found.
– Symptoms not found.

Fig. 1. The rounded pod symptom caused by an isolate from the New Juaben area of Ghana; photographed with a normal pod from a healthy tree.

PLATE 3. CACAO SWOLLEN SHOOT VIRUS

Fig. 2. Stem swellings caused by an isolate from Western Nigeria.

Mampong isolate consistently causes swellings, but has characteristically mild effects and the leaf symptoms are limited. That from Nkawkaw is also mild, but swellings are rare and flush leaves show a conspicuous red mottle.

Ashanti is now the major cacao area of Ghana but virus is less widespread than in the Eastern Province. Nevertheless, numerous scattered outbreaks occur and differences have been noted between some isolates. Viruses which do not protect against New Juaben or each other occur at Bosumtwe and Okerikrom, and they cause swellings but different leaf symptoms. Isolates of the New Juaben type have been obtained at Konongo and Sedi Nkawie, many miles from the nearest other known localities of this virus.

Infection is widespread in the Western Province and numerous isolates causing swellings have been distinguished by the leaf symptoms they cause (Todd, unpublished information). None of them protects against each other or against isolates from elsewhere (Table IIb). For example, at least four apparently unrelated isolates have been found near Wiawso, one of the few districts investigated in detail. The situation at nearby localities appears equally complex and this may be partly due to infection spreading into cacao from wild *C. chlamydantha* (see later section).

Scattered outbreaks occur in cacao in Trans-Volta and Togoland; some of them involving viruses of the Kpeve type. This causes an extensive transient red mottle and clearing or banding of the veinlets between the principal veins, but no swellings (Posnette, 1947a). Another virus from Peki causes swellings only.

At least some of the different isolates from Ghana differ in host range (Tinsley & Wharton, 1958). There are also differences in the ability of the various mealybug species to transmit certain isolates and *Ferrisiana virgata* (Ckll.) transmitted five, but not those from Kpeve and Nkawkaw (Posnette, 1950b).

Nigeria

The acreage of cacao in Nigeria is less than a quarter of the four million or more acres in Ghana, but infection is widespread in the Western Region. Indeed, control measures had to be abandoned in parts of Abeokuta and Ibadan Provinces, which are acting as increasingly dangerous foci of infection (Lister & Thresh, 1957b; Thresh, 1958c).

The Nigerian isolates that have been studied are from different parts of the abandoned areas, and from scattered outbreaks around them. Ibadan Province has received most attention and isolates from this area cause a range of symptoms comparable to that found in Ghana. Most isolates cause swellings, but they can be distinguished

by their leaf symptoms and that from Elepo is particularly mild in its effects. By comparison, the virulent Ikire virus may kill seedlings and causes severe leaf chlorosis, defoliation and often die-back. Other isolates from the Ibadan Native Administration farm and from Ilaro cause swellings but no obvious leaf symptoms. Swellings have not been found on plants infected with isolates from Abuku and Balogun, though these cause leaf symptoms of the type usually associated with swellings. The isolate from Alaparun is the most distinct of the isolates not causing swellings and resembles the one from Kpeve.

Protection tests show that isolates from Olanla and Ilesha are closely related, but others usually fail to protect against each other, or against viruses from Ghana and Ivory Coast (Table II). Differences between the Nigerian isolates have also been noted in host range (Tinsley & Wharton, 1958) and vector studies (Posnette, 1950b), and some isolates from Abeokuta Province were not transmitted by mealybugs.

Ivory Coast

Virus disease of cacao in Ivory Coast was first found in 1945 and swellings have been found at most of the outbreaks, associated with various leaf symptoms (Renaud, 1957). Transmission experiments confirmed that different viruses or strains were present and two were studied by Mangenot *et al.* (1946). Protection tests subsequently showed that one from Kongodia was related to an isolate from New Juaben. The virus from Sankadiokro resembled the one from Bosumtwe in Ashanti, although protection tests showed no close relationship with this virus and others from Kongodia, New Juaben and elsewhere in Ghana. Less detailed tests have been done with other isolates, but numerous symptomologically distinct ones have been found and some cause swellings only (Renaud, 1957).

Trinidad

Possible differences between viruses from different localities outside West Africa have received little attention, although Posnette (1944) observed that two isolates from Trinidad did not cause swellings but were clearly distinct from each other. The 'red mottle' virus was common in the Santa Cruz Valley and in Diego Martin and caused red vein banding followed by a limited translucent banding. By comparison the other so-called 'vein clearing' virus was present at Santa Cruz and the Maracas valley and caused extensive vein banding, often affecting the whole lamina. Affected tissues were usually translucent and the reticulate mosaic symptoms differed from the chlorotic banding caused by most West African isolates.

The symptoms caused by the two isolates vary with the indicator clones used and this led Baker & Dale (1947) to name them strains A and B. This provisional terminology is preferable, as there is no other evidence to justify separate names.

Ceylon

At least one isolate from Ceylon has been transmitted by mealybugs (Carter, 1956), but there have been no detailed comparisons with others from different areas. Swellings have recently been reported at some localities (Orellana & Peiris, 1957), although leaf mosaic was recorded some years ago (Peiris, 1953) and suspicious leaf symptoms even earlier (Urquhart, 1951).

THE DIFFERENCES BETWEEN ISOLATES FROM DIFFERENT TREES IN THE SAME LOCALITY

The differences between isolates from different trees in the same localities have received little attention. Nevertheless, trees in the same or nearby outbreaks may be infected by viruses differing considerably in the symptoms they cause and in their effects on growth. This situation occurs at Kpeve (Anon, 1948) and in the Western Province of Ghana (Anon, 1948), but has been most studied in the New Juaben area where some trees survived, although most had been killed by virulent virus. Some survivors had escaped or tolerated infection, but many contained mild strains which protected them from the effects of virulent ones (Posnette & Todd, 1951, 1955). Differences between viruses in the New Juaben outbreaks were also shown by coppicing and back-tests. Thus, most trees in the originally discovered outbreak of swollen shoot disease contained virulent strains, while many also contained mild ones, which sometimes occurred alone (Posnette & Todd, 1955). The mild strains differed from the typically virulent New Juaben isolates by causing inconspicuous leaf symptoms without swellings.

A single examination of outbreaks in the New Juaben area did not indicate the incidence of mild and virulent strains, which may cause similar symptoms at some stages of infection (Posnette & Todd, 1955). Furthermore, trees chronically infected with mild strains often seem healthy. Thus a virus is typical of an outbreak or locality only if it is the usual one obtained on testing an adequate sample of infected trees. This has not always been done and the choice of inoculum has sometimes been subjective and arbitrary. Consequently, laboratory cultures are not necessarily typical of an area and

laboratory selection may cause further divergence from the original field isolate.

The differences between isolates from adjacent outbreaks in Ivory Coast, Trinidad and Ceylon have not been investigated, but Nigerian experiments show that the Ghana situation is not unique. For example, isolates from different trees at Olanla, Offa Igbo and Araromi differed in the type and severity of the symptoms they caused. Similarly at Koroboto, monthly symptom records show that most trees produce swellings and conspicuous leaf mosaic. However, some trees produce only swellings, and others only mosaic symptoms, which may be very mild. These differences are partially due to differences in host reaction, but transmissions to seedlings usually reproduce the field symptoms (Thresh, 1958b).

Differences between isolates obtained from different trees in the same outbreak are apparently common and protection tests carried out in Ghana and Nigeria suggest that this is usually because related strains occur and not because of different viruses. Indeed, Bosomuosu and Mamfe in Ghana are the only localities from which different isolates have been obtained which fail to interprotect. The situation within restricted areas clearly differs from that between them, and is consistent with information on spread (Thresh, 1958a). Spread over great distances is rare and the probability of two outbreaks being infected from the same source decreases rapidly as the distance between them increases. Consequently, it is understandable that viruses from different areas are less closely related than viruses from the same area (cf. tomato black ring virus which also spreads slowly and not over great distances, Harrison, 1958).

P. njalensis can simultaneously transmit unrelated isolates (Posnette & Robertson, 1950) and probably related ones (Posnette & Todd, 1955). Thus the differences between isolates from nearby trees may arise by segregation from a mixed inoculum or by mutation.

THE DIFFERENCES BETWEEN ISOLATES SIMULTANEOUSLY INFECTING PARTICULAR TREES

Most trees in the New Juaben area are simultaneously infected with viruses which cause symptoms differing considerably in virulence and intensity (Posnette & Todd, 1951; 1955). For example, mild and severe strains were obtained from different shoots on the same coppiced stump and from different parts of intact trees. Similarly, distinct isolates have also been obtained from infected trees at Kpeve (Dale, 1957a) and by selection in laboratory transmissions using isolates

originally collected from individual trees at Ikire, Elepo, Asijere, Balogun, Aladie, Ilesha and Koroboto in Nigeria (Thresh, unpublished information).

Atypically mild strains often segregate during routine transmissions, particularly when these involve single mealybugs, and difficulty has been experienced in maintaining laboratory cultures. For example, isolates from severely affected trees at Ikire commonly kill seedlings infected as beans, but this ability is lost on successive transfers from the survivors. Similarly, isolates causing swellings but only limited leaf symptoms are often lost because routine laboratory passages favour the selection of readily transmitted variants causing obvious leaf symptoms. Unintentional selection may also explain the apparently anomalous results in growth experiments with isolates which apparently changed in virulence between experiments (Dale, 1956, 1958; Lister & Thresh, 1955).

Mild isolates from New Juaben usually protect plants from subsequent infection with virulent ones from the same or adjacent trees; similarly with the mild and severe strains from Ikire and Koroboto. This is the only information on the relationships between different isolates from the same source. Nevertheless, they are probably strains of the virus typical of the area; mixed infection being favoured by the perennial host and its considerable size. Even if new variants are rare, they can multiply for long periods in surviving trees. Moreover, particular strains may become dominant in certain branches and die-back or coppicing enables unusual variants to become dominant. Growing conditions and the spread of cacao viruses by mealybugs moving through the canopy also facilitate mixed infection (Thresh, 1958a), as trees may be in contact with several neighbours, depending upon spacing, age and condition. By contrast, infrequent spread by individual windborne mealybugs is less likely to result in mixed infections and probably results in segregation. This partly explains the differences between isolates from adjacent outbreaks and between different parts of large outbreaks.

THE RELATIONSHIPS BETWEEN VIRUSES FROM CACAO AND THOSE AFFECTING WILD HOSTS

Most of the Tiliales known to be susceptible to cacao viruses are indigenous to the forest areas of West Africa and four species have already been found naturally infected with viruses which mealybugs can transmit to cacao. Further tests will probably reveal other wild hosts, although it is often difficult to detect virus in tolerant species or inaccessibly large trees.

The understory tree *C. chlamydantha* is the most important of the indigenous hosts. It is common, up to 50 to the acre occurring in parts of the Western Province of Ghana, within and alongside cacao farms and in forest areas now being cleared for cacao. Surveys (Posnette *et al.*, 1950; Todd, 1951a; Attafuah & Tinsley, unpublished information) indicate that many trees are naturally infected with viruses similar to those in nearby cacao. This might indicate only that virus has spread from the crop, but infection in remote forest reserves suggests that the indigenous host was infected first. The spread of virus in *C. chlamydantha* was probably accompanied by mutation and segregation, which may explain the differences now found between the various western Province isolates.

C. chlamydantha occurs in Ghana only in the Western Province and infection has not been reported from Ivory Coast, Sierra Leone or Nigeria. Nevertheless, other hosts may occur in these areas and viruses which cause swellings in cacao occur in *Cola gigantea* var. *glabrescens* Brenan *et* Keay and the silk cotton, *Ceiba pentandra* Gaertn. The infected *C. gigantea* was a seedling near an outbreak in cacao in the Eastern Province of Ghana. Similarly, an infected *C. pentandra* seedling was found in an outbreak near Offa Igbo in Nigeria and the only infected tree was found in heavily infected cacao near Tafo. Infection may have been present from many years in these hosts, although their importance cannot be assessed.

Some evidence for the importance of wild hosts is provided by the occurrence of viruses in the baobab, *Adansonia digitata* L. in Ghana (Plate I, Figure 3). These cause symptoms quite different from swollen shoot (Attafuah & Tinsley, 1958). *A. digitata* is common in the savannah areas of West Africa, north of the lowland forest areas where cacao is grown and it is frequently planted in or around forest villages and on the Accra plains. Most of the known infected trees are remote from cacao, but one from Wusuta in Togoland (Dale & Attafuah, 1957) contained a virus similar to the one in nearby cacao. This suggests that cacao viruses of this type primarily infect baobab and that infection appears in cacao on rare occasions when mealybugs are carried from wild hosts. The virus from Alaparun in Nigeria is similar and, perhaps significantly, this locality is near the edge of the lowland rain forest alongside areas where baobabs are common.

THE RELATIONSHIPS BETWEEN THE VARIOUS ISOLATES OF VIRUS FROM CACAO

The Nigerian isolates from adjacent localities in Ibadan, Ijebu-Ode and Abeokuta Provinces, differ from all others found in Nigeria

or elsewhere. Indeed the one from Asalu in Abeokuta Province was first described as 'A new cacao virus' (Anon, 1948), and causes an initial necrotic reaction from which plants recover to show limited, distinctive leaf symptoms. Red vein banding, mottle or reticulate mosaic are not found on the leaves and swellings are not produced (Thresh, 1958b). The isolates causing necrosis are the only ones from cacao which have been adequately tested but not transmitted by mealybugs. They infect plants already containing mealybug-transmitted viruses and reciprocal infections are readily made. For these reasons isolates of the Asalu type are ascribed to a separate virus and the name *cacao necrosis* virus has been proposed (Thresh, 1958b).

In Nigeria, cacao necrosis virus appears to be confined to localities around Asalu, although the inconspicuous chronic symptoms may have been overlooked in the search for swollen shoot disease. Elsewhere, Posnette & Palma (1944) in Venezuela and Orellana & Peiris (1957) in Ceylon found trees showing symptoms similar to those caused by cacao necrosis virus, but were unable to make transmission tests.

The remaining isolates from West Africa are transmitted by mealybugs, and all cause transient red vein banding symptoms on the young leaves, usually followed by a chlorotic mottle or reticulate mosaic and swellings. Furthermore, all the isolates studied have a similar host range, apparently restricted to the Tiliales and Malvales. These similarities make sub-division difficult and equivocal, but at least three approaches are possible:

1. Protection tests may be taken to suggest that many distinct viruses occur.
2. Additional criteria may be used to reduce the number of apparently distinct viruses.
3. The numerous isolates may be ascribed to a single cacao swollen shoot complex, within which protection tests indicate relationships.

Related viruses do not always interfere with each other and there is little justification for classifying cacao viruses solely on protection tests. This would have few advantages and give an impression of heterogeneity amongst isolates causing the same disease. However, ascribing the many isolates, including ones which do not cause swellings, to a single swollen shoot complex is an unnecessarily broad classification. Thus attempts have been made to use additional criteria and Posnette (1947b) described four isolates from Ghana as strains of *Theobroma* Virus I, but recognised that the one from Kpeve caused sufficiently distinct symptoms to justify the synonym 'cacao

mottle leaf'. Tinsley (1955) and Attafuah (1957) retained this name for viruses which consistently fail to produce swellings on the roots or stems of infected plants, and used cacao swollen shoot only for isolates causing swellings. There are objections to this as the term cacao mottle leaf described only the Kpeve isolate, which causes very conspicuous red banding and mottle symptoms and a characteristic chlorotic mottle on mature leaves. These symptoms are caused by only some of the isolates which do not cause swellings. Others cause a reticulate mosaic similar to the ones usually associated with swellings and also differ from those of the mottle type in that they are transmitted by *F. virgata*.

Clearly, *cacao mottle leaf* virus was heterogenous as originally defined and we suggest that it should be restricted to isolates causing chlorotic mottle symptoms, but not swellings or a reticulate mosaic closely related to the principal veins. Thus it would include all the isolates from baobabs in Ghana, from cacao at Kpeve and other localities in Togoland and from Alaparun in Nigeria (Table III).

Different strains of cacao mottle leaf virus have been isolated from cacao and baobabs, although they cause such similar symptoms that the results of protection tests are equivocal (Attufuah & Tinsley, 1958). However, the Kpeve isolate superinfects plants already containing the one from Alaparun (Attafuah, 1957), indicating that some strains are more closely related than others.

Viruses transmitted by mealybugs and which cause a reticulate leaf mosaic cannot be ascribed to the cacao necrosis or cacao mottle leaf viruses. They usually cause swellings on roots or stems and with the apparent exception of the isolate from Nkawkaw in Ghana the ones which have been studied are transmitted by mealybug species including *F. virgata* but excluding *P. adonidum*. However, they cause a range of symptoms and host range studies, vector investigations and protection tests also show heterogeneity. Nevertheless, there is insufficient evidence for wholesale sub-division into distinct viruses with separate names. Consequently our provisional proposal is that the numerous isolates should be ascribed to a complex of *cacao swollen shoot* viruses. In the same way viruses which fail to protect against each other but cause curly top disease of sugar beet in the Americas are grouped together. Similarly with the tobacco necrosis viruses, some of which are serologically distinct.

Protection tests reveal that some isolates in the cacao swollen shoot complex are more closely related than others, and several groups occur which may eventually be distinguished as separate viruses. The components of each group are most conveniently referred to as strains, they protect against each other, but not against those in other groups. For example the numerous New Juaben strains and others

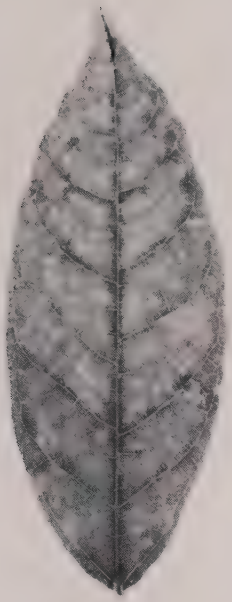


Fig. 1. Angular chlorotic flecks produced at the earliest stage of infection.

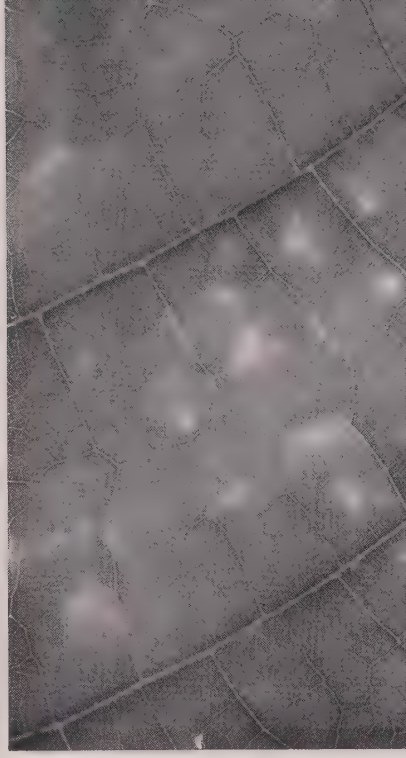


Fig. 2. Angular chlorotic flecks magnif

PLATE 4. CACAO SWOLLEN SHOOT VIRUS

Fig. 3. General chlorosis produced in the acute stage of infection with a virulent isolate.

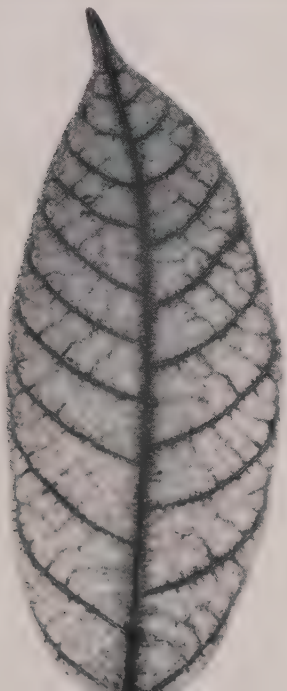


Fig. 4. General chlorosis magnified.

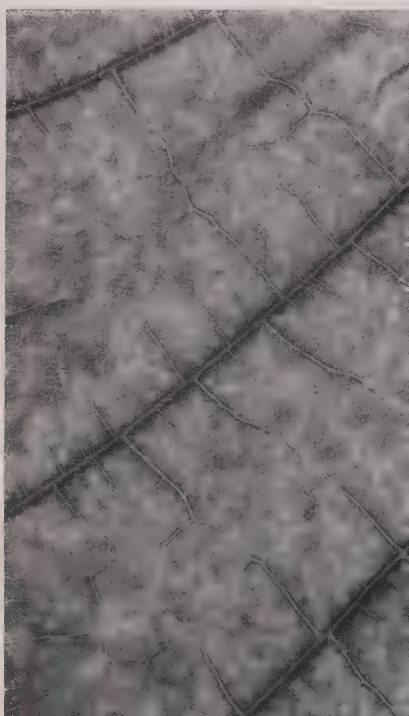


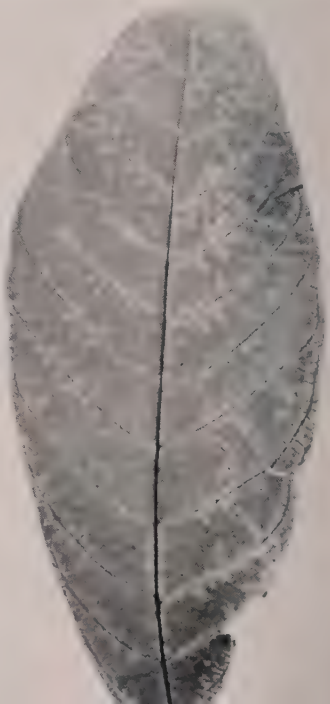
Fig. 1. Distortion and irregularly distributed vein banding.

Fig. 2. Speckled clearings caused by an isolate having mild effects.

PLATE 5. CACAO SWOLLEN SHOOT VIRUS

Fig. 3. Chlorotic banding of the veinlets along the third and fourth order veins. Chronic stage of infection.

Fig. 4. Chlorotic banding of the veinlets along the second, third and fourth order veins. Chronic stage of infection.



from Kongodia, Konongo and other localities in Ghana form one group and the strains from Ilesha and Olanla form a second. Further work may extend this grouping and indicate specific associations with indigenous hosts, although this approach is limited by their number.

There is no information on the behaviour of the Trinidad and Ceylon isolates in protection tests or on the range of hosts they infect. Furthermore, the symptoms they cause have not been compared with those caused under the same conditions by West African viruses and published descriptions were based on different seedlings and clones. Clearly there can at present be no valid assessment of the relationships between the viruses from West Africa and those from elsewhere, but the reticulate leaf symptoms and swellings found in Ceylon suggest the presence of the cacao swollen shoot viruses. Dale and Posnette (personal communications) have stressed the dissimilarity between the West African isolates and those from Trinidad and the characteristic translucent clearings and absence of swellings suggest that distinct viruses occur.

SUGGESTIONS FOR FUTURE WORK

Detailed investigations into swollen shoot disease have been in progress almost twenty years. Nevertheless, many aspects of cacao viruses require further investigation. In all future work it is suggested that greater attention should be given to the differences between isolates from adjacent localities and from the different parts of sampled trees. Greater attention should also be given to the selection of isolates and these should be given a locality name only when they are typical of an area. Moreover, repeated single insect transfers should be made to establish relatively stable cultures and increase the precision of laboratory experiments.

Symptom Expression

Typical isolates from different countries should be compared at one place, under similar conditions and on the same type of host. However, there are obvious quarantine difficulties in assembling viruses in a tropical country and the work could be done in glasshouses in temperate conditions.

It is suggested that symptoms should be described only on unpigmented Amelonado seedlings infected as beans, although further investigations may reveal clones of greater uniformity and sensitivity, to act as differential indicators.

Transmission

The repeated failure to transmit cacao viruses by sap inoculation should not prejudice further attempts using new techniques. Successful sap inoculation methods with cacao viruses would be a major advance, enabling additional criteria and perhaps serology to be used in classification.

Further attempts should be made to transmit cacao necrosis virus and to confirm and extend the results obtained with mealybug transmitted isolates. Ideally, laboratory cultures of mealybugs should be used and their ability to transmit the various isolates compared simultaneously. Particular attention should be given to experiments with what appear to be key species such as *F. virgata* and *P. adonidum* and the conditions under which they transmit the more important isolates.

Host Range Studies

Most of the commoner Tiliales indigenous to West Africa have already been tested for their susceptibility. Further work with these hosts may indicate relationships between the different strains and other wild hosts may be found. Particular attention should be given to the hosts already known to show differential susceptibility, and to the symptoms caused on the different hosts.

Further studies on the incidence and distribution of infected wild hosts could improve control measures and may provide data on virus relationships. The importance of *C. chlamydantha* and *A. digitata* is known, but there is less information on the viruses they contain. Further work is also required on the relationships between viruses from *A. digitata*, *C. chlamydantha* and other wild hosts and those present in cacao. Information on the incidence of infection in forest reserves and in cacao areas where little or no virus has been found within the crop would be of particular interest, and these studies should be extended outside West Africa.

Protection Tests

Interference between isolates is so rare that the available techniques apparently demonstrate only the closest relationships. Consequently it is suggested that further large scale tests should be delayed pending a detailed study of the protection phenomenon. Attention should be given to determine:

1. The most convenient size of test plant.
2. Whether the plants should be cut back or defoliated at any stage.

3. Whether mealybugs or grafts should be used for the inoculations.

4. The optimum interval between inoculations.

5. The suitability of mild strains for the first inoculum.

6. Whether observations on growth give evidence of relationship (cf. Holmes, 1956).

SUMMARY

In 1938, swollen shoot of cacao in Ghana was shown to be a virus disease. Virus diseases of cacao have subsequently been found in Ivory Coast, Nigeria, Trinidad and Ceylon and probably also in Colombia, Venezuela and Java. The general properties of the isolates found in the various outbreaks are summarised and the criteria available for classifying them are discussed and used to divide the ones from West Africa into three distinct groups, viz:

Cacao Necrosis Virus:

Acute necrotic stage of infection followed by recovery phase with limited leaf symptoms, but no swellings and no red vein banding or mottle on flush leaves.

Translucent distorted patches on hardened leaves, but no reticulate mosaic.

Apparently not transmitted by mealybugs, although naturally occurring outbreaks spread slowly.

Unrelated to other cacao viruses by protection tests.

Apparently restricted to adjacent localities in south west Nigeria.

Different isolates cause dissimilar symptoms and effects on growth.

Cacao Mottle Leaf Virus:

No marked distinction into acute and chronic phases of infection and no necrosis or swellings.

Extensive red vein banding and mottle on flush leaves.

Mottle symptoms on hardened leaves caused by clearing and banding of the veinlets between the main veins, but not alongside them.

Transmitted by several mealybug species, apparently excluding *Ferrisiana virgata* Ckll.

Host range apparently restricted to Tiliales and Malvales.

Unrelated to other cacao viruses by protection tests.

Apparently restricted to baobabs, *Adansonia digitata* L., north and

south of the main cacao areas in Ghana and to scattered outbreaks in the cacao of Trans-Volta and Togoland and near Alaparun in Nigeria.

Minor differences between isolates from baobabs and cacao.

Cacao Swollen Shoot Virus:

Usually a distinction into an acute stage of infection without necrosis and a relatively mild chronic phase, with swellings.

Red vein banding on flush leaves but rarely a general mottle.

Reticulate mosaic symptoms on at least some hardened leaves, caused by clearing and banding of the veinlets between and alongside the main veins.

Transmitted by several mealybug species usually including *F. virgata.*

Host range apparently restricted to the Tiliales and Malvales.

Unrelated to other cacao viruses by protection tests, which indicate that strains in the swollen shoot complex may be ascribed to groups, within which the strains inter-protect but between which they do not.

Widespread in most of the important cacao areas of Ivory Coast, Ghana and Nigeria.

Minor differences between isolates from the different parts of trees and outbreaks, with greater differences between typical isolates from different areas.

Not found in baobab but occur in other indigenous hosts.

There is so little information on the viruses infecting cacao in Trinidad, Ceylon and elsewhere that it is at present impossible to relate them to the viruses occurring in West Africa. However, those in Ceylon apparently fall within the cacao swollen shoot complex. It is suggested that at the earliest opportunity the viruses occurring in different parts of the world should be compared under the same conditions. Proposals are also made for further investigations into cacao viruses and their relationships.

ACKNOWLEDGEMENTS

We are indebted for much helpful criticism and advice during the preparation of this paper to A. Attafuah, F. C. Bawden, J. W. Blencowe, W. T. Dale, C. G. Johnson, J. Lamb, R. M. Lister, C. H. Martini, A. F. Posnette, and J. McA. Todd.

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